Gas Chromatographic Determination of Famphur and Its Oxygen Analog Residues in Bovine Milk, Blood, and Edible Tissues

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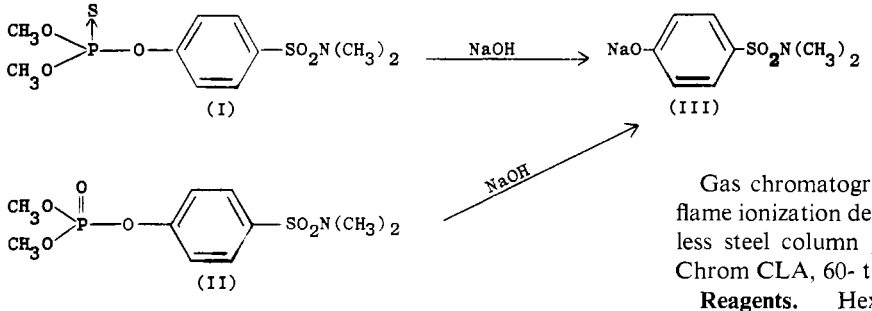
A gas chromatographic method for the determination of residues of famphur, *O,p*-(dimethylsulfamoyl)-phenyl *O,O*-dimethyl phosphorothioate, an organophosphate systemic insecticide, and its oxygen analog is described. Famphur and its oxygen analog are separated from each other by column chromatography. The two compounds are then hydrolyzed in alkaline medium to *p*-hydroxy-*N,N*-dimethylbenzenesulfonamide, which then reacts with hexamethyldisilazane. The resultant trimethylsilylether derivative is measured by gas chromatography. This method is satisfactory for the detection of famphur and oxygen analog residues in calf muscle, liver, and kidney down to levels of 0.05 p.p.m. and in milk to 0.005 p.p.m.

Famphur, *O,p*-(dimethylsulfamoyl)phenyl *O,O*-dimethyl phosphorothioate, an organophosphate systemic insecticide, is used to control cattle grub and louse infestations. It is either applied dermally as a 12.5% liquid concentrate or fed orally mixed with feed as a premix at levels to 4.5 mg. per pound of body weight.

The use of famphur for insect control required knowledge of the possible excretion of the insecticide and its major toxic metabolites in milk or the accumulation of these compounds in tissue.

Preliminary analytical investigations showed that famphur had a very low vapor pressure and could not be subjected to gas chromatography. The ultraviolet spectral characteristics, the exhibition of a strong absorption maximum at 232 mμ, made this technique practically impossible because of the extensive cleanup that would be involved.

Famphur (I) and its oxygen analog (II) are quantitatively hydrolyzed with sodium hydroxide to *p*-hydroxy-*N,N*-dimethylbenzenesulfonamide (HDMBS) (III).



The *p* - hydroxy - *N,N* - dimethylbenzenesulfonamide (HDMBS) sodium salt showed a strong absorption band at 265 mμ and, like the parent compound famphur, absorbs too low in the ultraviolet region to make direct ultraviolet measurement practical. The HDMBS was subjected to gas chromatography using polar and nonpolar liquid substrates and was either nonvolatile or failed to elute from the columns. Langer *et al.* (1958, 1960) and, more recently, other authors (Bentley *et al.*, 1963a, 1963b; Hedgley and Overend, 1960; Sen and McGeer, 1963) have reported on the trimethylsilylation of phenols, alcohols, and polyols. This technique yields a suitably volatile derivative of HDMBS and makes it possible to use a wide variety of liquid phases for its separation. A sample of HDMBS was subjected to trimethylsilylation and examined on XE-60, a polar phase, Dow Corning Silicone QF-1 (trifluoro propyl methyl silicone), DC-550 (phenylmethyl silicone), GE-SE-52 (methyl phenyl silicone), and Apiezon N (petroleum grease). Best results were obtained on Apiezon N columns.

This report deals with the development of a reproducible, specific, and quantitative method for the determination of famphur and its oxygen analog, *p*-hydroxy-*N,N*-dimethylbenzenesulfonamide dimethyl ester, in bovine milk and blood and in edible cattle tissues.

EXPERIMENTAL

Apparatus. Acetylation flasks, 25-ml. capacity, Bantamware, Kontes Catalog No. K-29425.

Adapters, Kontes Catalog No. K-27475.

Gas chromatograph, F & M No. 1609, equipped with flame ionization detector and 9-foot × 1/4-inch O.D. stainless steel column packed with 5% Apiezon N on Gas-Chrom CLA, 60- to 80-mesh.

Reagents. Hexamethyldisilazane reagent, Applied Science Laboratories, State College, Pa.

p - Hydroxy - *N,N* - dimethylbenzenesulfonamide, purified. Obtainable from Agricultural Division, American Cyanamid Co., Princeton, N. J.

Analytical Procedure. PREPARATION OF STANDARD SOLUTIONS. Prepare a volumetric solution of *p*-hydroxy-*N,N*-dimethylbenzenesulfonamide (HDMBS) in methanol to contain a concentration of 1 mg. per ml. Dilute this solution 1 to 200 with methylene chloride. Pipet 1, 2, 3, 4, and 5 ml. of the diluted solution into 25-ml. acetylation flasks, evaporate the solvent to dryness on a Rinco evaporator, and follow the procedure under Silylation.

SILYLATION. Disconnect the flask from the evaporator,

Agricultural Division, American Cyanamid Co., Princeton, N.J.

add exactly 0.4 ml. of hexamethyldisilazane to the flask, and stopper immediately. Tilt the flask and, with a slow rolling motion, distribute the solvent on the wall of the flask, being careful never to allow it to reach the ground-glass joint. Allow the reaction mixture to stand for a minimum of 1 hour. The above mixing procedure is performed at least three times during this 1-hour period.

CHROMATOGRAPHY PARAMETERS. Column: 9-foot × 1/4-inch O.D. stainless steel column of 5% Apiezon N on Gas Chrom CLA, 60- to 80-mesh. Flow rates: helium, 130 ml. per minute; hydrogen, 50 ml. per minute; air, 400 ml. per minute. Column temperature, 190° C. (isothermal); detector, 200° C.; injection port, 195° C. Attenuation, 2 or higher depending on peak size. Range, 10. Sample size, 5 μl. Chart speed, 1 inch per minute.

CALIBRATION CURVE. Inject 5-μl. aliquots of the strongest standard solution into the gas chromatograph until a constant response is obtained. This is necessary to condition the column. Inject 5-μl. aliquots of the remaining standards. The concentrations of these standards are 0.0625, 0.125, 0.1875, 0.25, and 0.3125 μg. per 5 μl., respectively.

Measure the peak areas with a planimeter. Plot the peak areas *vs.* concentrations on rectangular graph paper. A straight-line plot should be obtained in this concentration range.

Note. Daily preparation of a standard curve is not necessary. However, a standard must be run daily to check retention time and response. If the standard does not check with the curve, a new curve must be prepared.

PREPARATION AND EXTRACTION OF SAMPLES. Muscle, Liver, and Kidney. Weigh a 50-gram sample of ground tissue into a Waring Blendor or Omni-mixer jar. Add 300 ml. of acetonitrile and blend for 10 minutes. Filter the mixture by suction into a 500-ml. flask using a 7-cm. Büchner funnel fitted with a glass fiber filter. Wash the jar and solids with an additional 50 to 100 ml. of acetonitrile. Transfer the filtered extract quantitatively into a 1000-ml. round-bottomed flask, and evaporate the solution to dryness on a Rinco evaporator at 40° to 45° C. Transfer the residue into a 125-ml. separatory funnel using alternately three 10-ml. portions of methylene chloride and three 20-ml. portions of distilled water. Weigh 12 grams of sodium chloride, and add it to the separatory funnel. Stopper and shake gently for 1 minute. Allow the layers to separate and draw off the bottom layer (methylene chloride) into a 125-ml. Erlenmeyer flask. (If an emulsion forms at this stage, transfer the emulsion to a centrifuge bottle and centrifuge at 1500 r.p.m. for 5 minutes. The bottom layer can then be drawn off by pipet.) Add 20 ml. of methylene chloride to the original 1000-ml. round-bottomed flask, stopper, and shake vigorously. Pour this wash into the separatory funnel containing the aqueous phase and shake gently as before. Draw the bottom layer off into the Erlenmeyer flask. Repeat the extraction with an additional 20-ml. portion of methylene chloride and combine the methylene chloride extracts.

Prepare an alumina column as follows: Place a small plug of glass wool at the bottom of the 10 × 250 mm. column. Add 50 ml. of petroleum ether to the column. Measure 10 ml. of alumina in a graduated cylinder and pour slowly into the column. After the alumina is settled, draw off the petroleum ether to within 5 mm. of the top of the packing. Place a 250-ml. round-bottomed flask under the column and add the combined methylene chloride extracts to the column. Wash the Erlenmeyer flask with 10 ml. of methylene chloride, and add it to the column. Allow the solution to percolate through the column. Wash the column with three additional 20-ml. portions of methylene chloride, collecting this in the same flask (fraction A).

Place another 250-ml. round-bottomed flask under the column, and elute the column with 100 ml. of a 20% methanol solution in methylene chloride (fraction B). Evaporate the eluates (fractions A and B) to dryness on a Rinco evaporator (bath temperature 35° to 40° C.). Add 10 ml. of 1*N* sodium hydroxide to the residues, place the flasks in a water bath at 55° C., and heat at this temperature for 45 minutes. Cool the flasks containing the hydrolyzates to room temperature.

Prepare two Celite (diatomaceous silica products) filter mats as follows: With 2 to 3 grams of Celite 545, make a thick slurry with a few milliliters of distilled water. Pour the slurries into separate fine-porosity, sintered glass funnels, place the funnels on 125-ml. suction flasks, and apply suction. Do not allow the mats to dry completely. Filter the alkaline hydrolyzate solutions (fractions A and B) through the mats. Wash the flasks with small portions of 1*N* sodium hydroxide, using a total of 20 ml. Transfer the filtrates to 125-ml. separatory funnels using several small portions of distilled water for washing. Add 20 ml. of methylene chloride to the separatory funnels, stopper, and shake gently for 1 minute. Allow the layers to separate, and draw off the bottom layers and discard. Add 10 ml. of 6*N* sulfuric acid to the solutions, swirl to mix, and extract the solutions with three 20-ml. portions of methylene chloride, combining the extracts of each fraction in separate 100-ml. round-bottomed flasks. (The acidity of the aqueous solutions should be approximately pH 1 on universal indicator paper.)

Evaporate the solutions to dryness on a Rinco evaporator. Quantitatively transfer the residues remaining in the flasks into 25-ml. acetylation flasks using four 3-ml. portions of methylene chloride. Connect the flasks to an evaporator and evaporate to dryness (water bath at 35° to 40° C). Proceed as described under Silylation and Gas Chromatography.

Calculations. From the standard curve, obtain the concentration of *p*-hydroxy-*N*,*N*-dimethylbenzenesulfonamide corresponding to the sample peak area and calculate parts per million famphur and its oxygen analog as follows:

$$\text{P.p.m. famphur (fraction A)} = \frac{\text{concn. from calibration curve} \times 80 \times \frac{\text{attenuation}}{2} \times 1.618}{\text{sample weight}}$$

$$\text{p.p.m. oxygen analog (fraction B)} = \frac{\text{concn. from calibration curve} \times 80 \times \frac{\text{attenuation}}{2} \times 1.538}{\text{sample weight}}$$

Table I. Recovery of Famphur and Oxygen

Famphur Added, μg.	Levels, P.P.M.	Oxygen Analog Added, μg.	Levels, P.P.M.	Recovery, % Famphur	Recovery, % Oxygen analog
		Muscle 0.008 ± 0.008[b]			
5.0	0.1	...	...	86	...
5.0	0.1	...	...	94	...
5.0	0.1	...	...	101	...
5.0	0.1	5.5	0.11	80	97
5.0	0.1	5.5	0.11	86	59
5.0	0.1	5.5	0.11	89	101
...	...	5.5	0.11	...	98
10.0	0.2	...	...	99	...
10.0	0.2	...	...	85	...
10.0	0.2	11.0	0.22	79	70
10.0	0.2	11.0	0.22	85	89
25.0	0.5	...	...	74	...
30.0	0.6	...	...	62	...
30.0	0.6	...	...	105	...
30.0	0.6	11.0	0.22	75	79
30.0	0.6	11.0	0.22	87	83
		Liver 0.016 ± 0.024[b]			
2.5	0.05	...	...	53	...
2.5	0.05	...	...	93	...
2.5	0.05	...	...	75	...
2.5	0.05	...	...	112	...
5.0	0.1	...	...	105	...
5.0	0.1	5.5	0.11	144	69
5.0	0.1	5.5	0.11	96	108
10.0	0.2	...	...	108	...
50.0	1.0	25.0	0.5	86	93
50.0	1.0	25.0	0.5	88	75
50.0	1.0	...	...	97	...
		Kidney 0.024 ± 0.014[b]			
5.0	0.1	5.5	0.11	106	77
5.0	0.1	5.5	0.11	90	64
10.0	0.2	11.0	0.22	86	59
10.0	0.2	11.0	0.22	90	65
20.0	0.4	...	...	90	...
20.0	0.4	...	...	84	...
25.0	0.5	...	...	98	...
...	...	22.0	0.44	...	69
		Fat 0.008 ± 0.002[b]			
2.5	0.05	...	...	72	...
5.0	0.1	5.0	0.1	91	74

[a] All recoveries corrected for average control value.
[b] Mean and standard deviation for replicate control determinations.
[c] Control = NM, not measured with planimeter; less than 2 planimeter units by visual observation.

1.618 and 1.538 are factors for converting the sulfonamide to famphur and oxygen analog, respectively.

Fat. Weigh 50 grams of ground fat and transfer it to a Waring Blendor. Add 200 ml. of methylene chloride and blend at medium speed for 1 to 2 minutes. Add 10 grams of Celite 545 to the sample. Blend for 1 minute. Filter the sample with suction using a Büchner No. 2 porcelain funnel fitted with a glass fiber filter paper. Rinse the blender jar and cake with two 25-ml. portions of methylene chloride. Quantitatively transfer the filtrate to a 500-ml. round-bottomed flask. Evaporate the filtrate on a Rinco evaporator until there is no longer any odor of methylene chloride. Add 100 ml. of petroleum ether to the round-bottomed flask. If the sample solidifies, gently warm the flask and contents under a warm water tap and, with a swirling motion, mix until solids are completely dissolved. Quantitatively transfer the solution to a 500-ml. separatory funnel. Wash the flask with 50 ml. of petroleum ether and add to the separatory funnel. Rinse the flask with 50 ml. of water-acetonitrile solvent (1 to 4) and add to the separatory funnel. Shake the contents of the separatory funnel for 1 to 2 minutes. Allow the layers to separate. Draw off the acetonitrile layer (bottom layer) into a 250-ml. round-bottomed flask. Extract the petroleum ether

Analog from Bovine Tissues, Milk, and Blood[a]

Famphur Added, μg.	Levels, P.P.M.	Oxygen Analog Added, μg.	Levels, P.P.M.	Recovery, % Famphur	Recovery, % Oxygen analog
5.0	0.1	5.0	0.1	108	64
5.0	0.1	5.0	0.1	75	50
5.0	0.1	5.0	0.1	73	48
10.0	0.2	10.0	0.2	85	68
10.0	0.2	10.0	0.2	84	75
10.0	0.2	10.0	0.2	117	61
25.0	0.5	25.0	0.5	72	56
25.0	0.5	25.0	0.5	94	68
50.0	1.0	...	...	91	...
		Milk			
		Control = NM[c]			
5.0	0.025	...	...	65	...
4.8	0.024	5.0	0.025	75	84
10.0	0.05	...	...	86	...
10.0	0.05	...	...	82	...
9.6	0.048	10.0	0.05	73	62
14.3	0.072	15.0	0.075	71	70
19.1	0.096	20.0	0.10	74	65
19.1	0.096	20.0	0.10	103	78
25.0	0.125	...	...	89	...
25.0	0.125	...	...	84	...
25.0	0.125	...	...	95	...
...	...	25.0	0.125	...	78
50.0	0.25	...	...	93	...
50.0	0.25	...	...	102	...
50.0	0.25	...	...	96	...
100.0	0.5	...	...	112	...
100.0	0.5	...	...	96	...
100.0	0.5	...	...	99	...
100.0	0.5	...	...	72	...
100.0	0.5	...	...	86	...
25.0	1.25	...	...	93	...
		Blood			
		Control = NM[c]			
250.0	2.5	...	...	97	...
250.0	2.5	...	...	79	...
250.0	2.5	...	...	86	...
250.0	2.5	...	...	77	...
100.0	1.0	...	...	68	...
100.0	1.0	...	...	70	...
50.0	0.5	...	...	54	...
50.0	0.5	...	...	75	...
50.0	0.5	...	...	54	...
25.0	0.25	...	...	66	...
25.0	0.25	...	...	77	...
25.0	0.25	...	...	79	...
10.0	0.1	...	...	73	...
10.0	0.1	...	...	101	...
5.0	0.05	...	...	58	...
5.0	0.05	...	...	82	...

layer twice more with 50-ml. portions of aqueous acetonitrile solvent. Add each extraction to the 250-ml. round-bottomed flask. (The fat may tend to crystallize during the partitioning steps; if so, gently warm the separatory funnel and contents under a warm water tap until they dissolve.)

Evaporate the acetonitrile solution on a Rinco evaporator at 40° to 45° C. to a volume of 15 to 20 ml. Transfer the sample to a 125-ml. separatory funnel with the aid of 40 ml. of distilled water (total volume should be about 60 ml.). Add 12 grams (10 ml.) of sodium chloride to the separatory funnel, stopper, and shake until completely dissolved. Add 30 ml. of methylene chloride to the separatory funnel, stopper, and shake moderately for 1 minute. Draw off the methylene chloride layer (lower layer) into a 125-ml. Erlenmeyer flask. Extract the aqueous layer twice more with 20-ml. portions of methylene chloride, and combine the washes with the first extraction in the Erlenmeyer flask.

Proceed as described under Preparation and Extraction of Samples, under Muscle, Liver, and Kidney, beginning with "Prepare an alumina column," etc.

Milk. Acidify 200 ml. of milk with 3 ml. of 6*N* hydrochloric acid in a 2-quart wide-mouthed jar or bottle.

Add 800 ml. of hexane–methylene chloride solvent (1 to 4) and shake for 25 minutes on a reciprocating shaker at high speed. Using a 3- to 4-inch funnel, slowly pour the two-phase solution into a 1000-ml. separatory funnel. Allow the layers to separate and let stand for about 10 to 15 minutes. Slowly draw off the lower solvent layer into a 1000-ml. graduated cylinder and record the volume. Transfer the solvent layer into a 1000-ml. round-bottomed flask and evaporate the extract on a Rinco evaporator using a water bath set at 35° to 40° C. (alternatively, pour the extract into a 1000-ml. beaker and allow to evaporate overnight under the hood). Dissolve the residue remaining with 50 ml. of petroleum ether saturated with acetonitrile and transfer to a 125-ml. separatory funnel. Wash the flask with two 25-ml. portions of acetonitrile saturated with petroleum ether and add the wash to the separatory funnel. Shake the contents of the funnel moderately for 1 minute. Allow the layers to separate and draw off the lower layer (acetonitrile) into a 100-ml. round-bottomed flask. Repeat the washing procedure with a second 25-ml. portion of acetonitrile, combining the washings in the 100-ml. round-bottomed flask.

Evaporate the acetonitrile to dryness on a Rinco evaporator or until there is no more odor of acetonitrile. (Keep the water bath at 35° to 40° C.) Continue as described under Muscle, Liver, and Kidney, beginning with "Transfer the residue into a 125-ml. separatory funnel using alternately three 10-ml. portions of methylene chloride," etc.

Calculations:

$$\text{P.p.m. famphur (fraction A)} = \frac{\text{concn. from calibration curve} \times 80 \times \frac{\text{attenuation}}{2} \times 1.618}{\frac{\text{volume of sample}}{\text{volume of solvent added}} \times \text{volume of solvent recovered}}$$

$$\text{P.p.m. oxygen analog (fraction B)} = \frac{\text{concn. from calibration curve} \times 80 \times \frac{\text{attenuation}}{2} \times 1.538}{\frac{\text{volume of sample}}{\text{volume of solvent added}} \times \text{volume of solvent recovered}}$$

1.618 and 1.538 are factors for converting the sulfonamide to famphur and oxygen analog, respectively.

Blood. Proceed as described above for milk, but use 400 ml. of solvent and 100 ml. of blood containing 1.5 ml. of 6*N* hydrochloric acid.

Recovery Determinations. The tissue used in gathering all of the control and recovery data (Table I) was obtained from animals of known history. Muscle, liver, kidney, and fat were fortified with methylene chloride solutions of famphur and its oxygen analog, while milk and blood samples were fortified with aqueous solutions containing a small amount of methanol.

A 50-gram sample of tissue was used for all determinations of recovery and control values except milk and blood where 200- and 100-ml. samples were used, respectively. Recovery values obtained on samples fortified with 2.5 μg. of famphur and its oxygen analog resulting in more than 5 planimeter units are quantitative. However, the detection of less than the above concentration was semiquantitative because of the inability to measure accurately 1 to 4 planimeter units.

Feeding Studies. Three feeding experiments were designed to follow the rate of disappearance of famphur and its oxygen analog from calf tissues or milk. Data pertaining to feeding schedules, withdrawal times, or sacrifice dates, and residue values for the experiments are presented in Tables II and III.

Analysis of Tissues. The results of the experiments, summarized in Table II, show that all calf tissues are free of famphur and oxygen analog residues at 2 and 4 days post-medication when fed 1.5 and 4.5 mg. per pound body weight, respectively, for the periods of time designated in experiments A and B. The feeding of 3.3 mg. of famphur per kilogram of body weight per day to lactating cows for 90 days shows trace residues of famphur in milk from two of the three animals on the day of withdrawal and no detectable residues (< 0.005 p.p.m.) through the next four milkings (Table III).

DISCUSSION OF ANALYTICAL PROCEDURE

Investigations were carried out to determine the optimum conditions for the hydrolysis of famphur to *p*-hydroxy-*N*,*N*-dimethylbenzenesulfonamide (HDMBS) using various temperature and alkali concentration conditions. Hydrolysis was relatively slow at room temperature in either the aqueous or alcoholic-aqueous media using 1*N* sodium hydroxide or lower alkali concentrations. Complete hydrolysis is reached at the end of 30 minutes when famphur is hydrolyzed in 1*N* sodium hydroxide at 55° C. However, to allow for any margin of error, a hydrolysis time of 45 minutes is recommended. The completion of hydrolysis was determined by comparing the ultraviolet spectra in regions of 330 to 220 mμ of a hydrolyzed solution of purified famphur and famphur oxygen analog with that of purified HDMBS in a final concentration of 0.2*N* sodium hydroxide. Using a wavelength of 266.5 mμ absorption maximum for HDMBS, Beer's law studies made on the hydrolyzed solutions of famphur and famphur oxygen analog showed linearity and complete hydrolysis at all concentrations investigated (5 to 20 μg. per ml.).

Silylation of HDMBS was first carried out as described by Bentley *et al.* (1963a) by reacting the compound with hexamethyldisilazane (HMDS) in pyridine in the presence of trimethylchlorosilane (TMCS). There were two disadvantages in using these conditions for residue analysis. The use of pyridine introduced an additional diluent to the final solution causing a decrease in sensitivity of the method, and the presence of TMCS resulted in the formation of solids (ammonium chloride) which caused clogging of the syringe needle.

Investigations on the reaction of HDMBS in equal volumes of HMDS-TMCS in the absence of pyridine and finally in HMDS alone resulted in the same response as that run in the presence of pyridine. The time required for completion or maximum silylation was determined by reaction of 20 μg. of purified HDMBS with 100 μl. of HMDS for 5, 50, and 70 minutes. Response for all time intervals was the same, indicating complete or maximum silylation in 5 minutes. Silylation of HDMBS under the same conditions in the presence of tissue background re-

Table II. Famphur and Oxygen Analog Residues (P.P.M.)[a] in Cattle Tissues from Calves Fed Famphur

Calf No.	Days Post-medication	Muscle		Fat		Liver		Kidney	
		Famphur	Oxygen analog	Famphur	Oxygen analog	Famphur	Oxygen analog	Famphur	Oxygen analog
		1.5 Mg./Lb. Body Weight							
1 (control)	0	0.02	0.02	0.04	0.02	0.02	0.02	0.03	0.01
5	0	<0.05	<0.05	0.05	<0.05	0.30	0.16	<0.05	<0.05
6	0	<0.05	<0.05	0.22	<0.05	0.22	<0.05	<0.05	0.07
7	0	<0.05	<0.05	0.56	<0.05	0.61	0.11	<0.05	0.21
2 (control)	1	0.01	0.02	0.01	0.03	0.03	0.01	0.02	0.01
8	1	<0.05	<0.05	0.38	<0.05	<0.05	<0.05	<0.05	<0.05
9	1	<0.05	<0.05	0.29	<0.05	<0.05	<0.05	<0.05	<0.05
10	1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
3 (control)	2	0.02	0.02	0.03	0.02	0.02	0.01	0.01	0.02
11	2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
12	2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
13	2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
4 (control)	4	0.03	0.03	0.03	0.01	0.03	0.03	0.02	0.04
14	4	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
15	4	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
16	4	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		4.5 Mg./Lb. Body Weight							
1 (control)	0	0.03	0.03	0.03	0.04	0.03	0.02	0.05	0.04
5	0	0.11	<0.05	1.60	<0.05	5.60	0.34	0.13	0.13
6	0	0.31	<0.05	1.50	0.23	1.70	0.42	0.49	0.17
7	0	0.09	<0.05	0.61	<0.05	2.00	0.50	0.37	0.19
2 (control)	2	0.03	0.05	0.03	0.04	0.05	0.02	0.01	0.03
8	2	<0.05	<0.05	0.07	<0.05	0.05	<0.05	<0.05	<0.05
9	2	<0.05	<0.05	0.17	<0.05	0.11	<0.05	<0.05	<0.05
10	2	<0.05	<0.05	0.19	<0.05	0.07	<0.05	<0.05	<0.05
3 (control)	4	0.02	0.04	0.03	0.02	0.01	0.02	0.01	0.03
11	4	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
12	4	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
13	4	<0.05	<0.05	<0.05	<0.05	0.05	0.05	0.05	0.05
4 (control)	8	0.03	0.01	0.03	0.02	0.04	0.02	0.02	0.02
14	8	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
15	8	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
16	8	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

[a] All controls are apparent values and all samples are corrected for average controls.

Table III. Apparent Famphur and Oxygen Analog Residues (P.P.M.) from Cows Fed 3.3 Mg. Famphur per Kg. Body Weight for 90 Days

Post-Medication and Sample Hour	Cows on Treatment						Controls					
	1		2		3		4		5		6	
	Famphur	Oxygen analog	Famphur	Oxygen analog	Famphur	Oxygen analog	Famphur	Oxygen analog	Famphur	Oxygen analog	Famphur	Oxygen analog
0 day, A.M.	0.025	NM[a]	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
0 day, P.M.	0.018	NM	0.023	NM	NM	NM	NM	NM	NM	NM	NM	NM
1 day, A.M.	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
1 day, P.M.	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
2 days, A.M.	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
2 days, P.M.	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM

[a] NM = Not measured with planimeter. Less than 2 planimeter units by visual observation = <0.005 p.p.m.

sulted in low recoveries, indicating insufficient reagent. However, increasing the volume of HMDS to a minimum of 400 μl. gave quantitative recoveries and a response that was adequate for determining residues at the 0.05-p.p.m. level using 50 grams of tissue. A 1-hour silylation time is used in the procedure as a safety factor.

The gas chromatography of the trimethyl silyl ether (TMS) of HDMBS was studied on columns containing liquid phases of Dow Corning QF-1 (trifluoropropyl methyl silicone), DC-550 (phenylmethyl silicone), GE SE-52 (methyl phenyl silicone), and Apiezon N (petroleum grease) coated on Gas Chrom CLA, of which the last two were the most suitable. However, best resolution of the TMS derivative of HDMBS from tissue background peaks was accomplished with a 9-foot × 1/4-inch O.D. 5% Apiezon N (petroleum grease) on Gas Chrom CLA column using the gas chromatography conditions outlined in the analytical procedure. Peak retention times were reproducible, varying no more than 0.3 minute in a day's run. This proved useful in identifying sample peaks in the presence of excessive background. Retention times were affected considerably by changes in column temperature; this

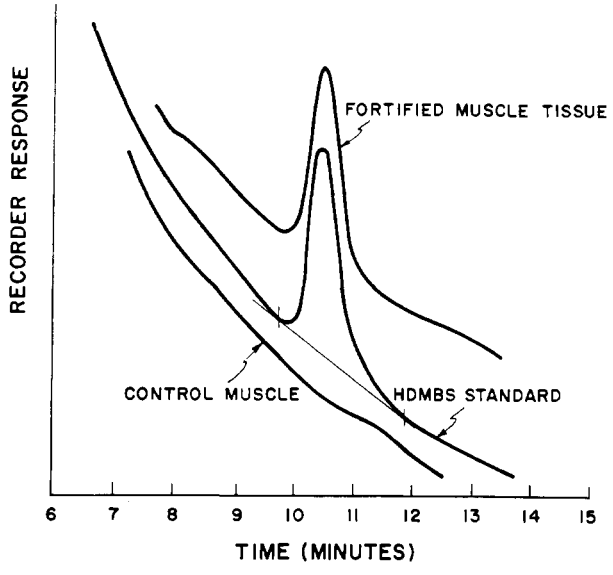


Figure 1. Chromatography of TMS derivative of HDMBS standard, fortified muscle tissue, and control tissue

factor could be used in resolving peaks when necessary.

In running tissue samples, it was observed that a white solid material gradually built up on the collector electrode and walls of the detector compartment. This was believed to be silylated derivatives of tissue material. The electrode and compartment required periodic cleaning because of this. Column life was a factor in the quality of the chromatograms. After prolonged use of the Apiezon (petroleum grease) columns, peaks broadened and tailing increased. This effect was noted after approximately three weeks of continuous daily use. A column showing these signs should be replaced. Results were calculated by comparison of sample peaks with a standard curve prepared from HDMBS standards. Because of sloping base lines, integrators are useless in computing peak areas. Areas are measured by drawing a base line tangent to the curve and measuring the area enclosed by this line and the peak by means of a planimeter (Figure 1).

Activated alumina was used to separate famphur from the oxygen analog and both compounds from tissue background. Initial handling and extraction of the various tissues differed considerably, but all were brought to a common point just preceding column chromatography. At this point, the dry residues were dissolved in methylene chloride which was then saturated with water. Upon percolation of the methylene chloride solution through the alumina column, the oxygen analog is retained with the bulk of the tissue background, and the famphur comes through with the solvent. Additional reagent solvent is passed through the column to ensure complete recovery of the famphur. The adsorbed oxygen analog is eluted from the column with 20% methanol in methylene chloride. Both fractions carried through the analytical procedure yield chromatograms that are free of tissue background. Figure 1 presents chromatograms of a standard famphur solution, control muscle for apparent famphur and oxygen analog, and a sample of fortified muscle carried through the analytical procedure.

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LITERATURE CITED

Bentley, R., Sweeley, C. C., Makita, M., Wells, W. W., *Biochem. Biophys. Res. Commun.* **11,** 14 (1963a).
Bentley, R., Sweeley, C. C., Makita, M., Wells, W. W., *J. Am. Chem. Soc.* **85,** 2497 (1963b).
Hedgley, E. J., Overend, W. G., *Chem. Ind. (London)* **1960,** p. 378.
Langer, S. H., Connell, S., Wender, I., *J. Org. Chem.* **23,** 50 (1958).
Langer, S. H., Pantages, P., Wender, I., *Chem. Ind. (London)* **1960,** p. 1664.
Sen, N. P., McGeer, P. L., *Biochem. Biophys. Res. Commun.* **13,** 390 (1963).

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